Transition of Methotrexate Polyglutamate Drug Monitoring Assay from Venipuncture to Capillary Blood-Based Collection Method in Rheumatic Diseases

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Objective: Methotrexate (MTX) polyglutamate (MTXPG$_3$) levels from isolated red blood cells (RBCs) collected by venipuncture have clinical utility in guiding MTX dosing for patients with rheumatoid arthritis (RA). Our objective was to transition this RBC-based therapeutic drug monitoring (TDM) assay to dried capillary blood collected by fingerstick.

Methods: Patients with RA treated with MTX were enrolled. Specimens were collected by fingerstick (volumetric absorptive microsampler) and venipuncture to measure MTXPG$_3$ from dried capillary blood, total venous blood, and isolated RBCs. MTXPG$_3$ levels from dried capillary blood were measured using LC-MS/MS, converted to RBC equivalent (nmol/L), and compared with those from isolated RBCs (reference method). Following transition to fingerstick collection, comparability in the distributions of dried capillary and venipuncture-based RBC MTXPG$_3$ levels was assessed using the Kolmogorov–Smirnov (K-S) test.

Results: Intraday and interday precision ranged from 2.0% to 10.9% and 3.1% to 10.8%, respectively, at MTXPG$_3$ concentrations ranging from 5 to 100 nmol/L. In 106 participants treated with MTX, MTXPG$_3$ levels from total venous and dried capillary blood were comparable [slope = 0.97 (95% CI, 0.92–1.03); R^2 = 0.92]. Dried capillary blood MTXPG$_3$ converted to RBC equivalent was similar to levels from isolated RBCs (30 ± 18 nmol/L vs 33 ± 19 nmol/L; n = 106). After implementation in the clinical laboratory, RBC equivalents MTXPG$_3$ from the fingerstick method were similar to levels from venipuncture [39 ± 22 nmol/L (n = 825) vs 39 ± 24 nmol/L (n = 47 935)] (K-S test *P* = 0.09). Underexposure to MTX (MTXPG$_3$ ≤5 nmol/L RBCs) was detected in 7.0% and 8.5% patient specimens collected using the fingerstick and venipuncture methods, respectively.

Conclusion: Capillary blood MTXPG$_3$ levels can be used to guide MTX dosing in TDM practice.

IMPACT STATEMENT

We have developed a dried capillary blood-based LC-MS/MS method to quantify long-chain methotrexate polyglutamate (MTXPG) in adult patients with rheumatoid diseases. MTXPG$_3$ measured in dried capillary blood collected by fingerstick was expressed as red blood cell equivalent MTXPG$_3$ by applying a conversion factor determined during validation. After 1 year of implementation in the clinical laboratory, similar mean MTXPG$_3$ levels and distributions were observed compared with our previously validated method for quantifying MTXPG$_3$ in isolated red blood cells. Surveys collected from each patient enrolled during validation revealed that the fingerstick collection method was convenient and noninferior to venipuncture. This method could increase adoption of routine methotrexate therapeutic drug monitoring, especially in patients for whom a blood draw is difficult, and in collection settings such as small community rheumatology offices where a licensed phlebotomist may be unavailable.

Methotrexate (MTX)[4] is an antifolate drug and pivotal frontline treatment for patients with rheumatoid arthritis (RA) (1). The pharmacological effects of this prodrug are dependent on a folylpolyglutamate mediated γ-linked sequential addition of glutamic acid residues on the para-aminobenzoic acid moiety of MTX to produce long-chain methotrexate polyglutamates (MTXPGs). The inhibitive effects of these MTXPGs on key enzymes involved in folate and novo purine biosynthesis are several orders of magnitude more potent than the effects of the parent MTX (2–4). As such, long-chain MTXPGs are key objective measures for assessing MTX exposure and effectiveness in autoimmune rheumatic diseases.

A low-dose weekly MTX-based regimen is the cornerstone disease-modifying antiarthritic drug treatment (5) and is effective in controlling disease activity in many RA patients (6). However, unusually poor activation to long-chain MTXPGs (MTXPG$_3$) or underexposure secondary to poor compliance can limit achievement of a robust clinical response (7, 8) in some patients, although it is clear that other mechanisms unrelated to pharmacokinetics are also relevant (9). Many investigators have proposed that the determination of intracellular MTXPGs from red blood cells (RBCs) can objectively help detect underexposure as a possible underlying cause of an inadequate exposure to MTXPGs (10–12), and commercial laboratories have been offering therapeutic drug monitoring (TDM) of low-dose MTX therapy for a decade.

TDM of low-dose weekly MTX regimens have historically relied on RBCs isolated from anticoagulated blood specimens collected by venipuncture and quantification of long-chain MTXPGs (specifically, MTXPG$_3$) by liquid chromatography coupled with various detection methods, including tandem mass spectrometry (13–15). It is well established that collection of capillary blood from fingerstick is a key alternative to venipuncture and may ease the burden of specimen collection. Recently, a volumetric absorptive microsampling (VAMS) device that addresses some of the major limitations of dried blood spot card has been developed (16) and applied to TDM of hydroxychloroquine (17) and other medications (18, 19). Rather than capturing blood on filter paper, the VAMS device (Mitra^{TM}, Neoteryx) uses an absorptive polymer, thus ensuring a consistent volume of blood is collected. In this study, we sought to transition the RBC MTXPG$_3$ assay from venous blood collected by venipuncture to capillary blood collected by fingerstick.

MATERIALS AND METHODS

Fingerstick and venipuncture specimen collection in RA patients

Adult patients diagnosed with RA undergoing low-dose MTX therapy (17 ± 5 mg/week for at least 2 months) in the San Diego, California, metropolitan area were enrolled between November 2015 and September 2016. Informed consent was collected for all patients, and the study protocol was approved by the central review board Solutions Institutional Review Board.

Capillary blood was collected by fingerstick (using BD Microtainer™ Contact-Activated Lancet) on VAMS (Mitra 10-μL microsampling device in clamshell, Neoteryx) tips per detailed instructions provided to the medical staff. On collection of capillary blood, the microsamplers were dried overnight, sealed in a biohazard bag with a silica desiccant pouch (Dry and Dry), and shipped to the clinical

laboratory using standard priority mail. For each participant, a paired venous blood specimen was also collected by venipuncture in EDTA-containing tubes (10 mL) and shipped overnight using a transportation kit containing a frozen coolant cartridge.

On receipt in the TDM laboratory, VAMS specimens were stored at ambient temperature for 3 days after specimen collection before processing (as described below). RBCs were isolated from venous blood on the day of receipt (1 day after collection) as described (13) and stored at −80 °C. An aliquot of whole venous blood was also stored at −80 °C to quantify total MTXPG$_3$. Hematocrit from venous blood (known to be interchangeable with capillary blood hematocrit) (20) was measured using 75-mm microhematocrit capillary tubes. A patient survey comparing the fingerstick and venipuncture methods was collected for each patient.

Sample treatment procedure of dried capillary blood, venous blood, and isolated RBCs

On receipt of capillary blood specimens, trained technologists inspected VAMS tips for specimen integrity and appropriate filling. To extract MTXPG$_3$ from dried VAMS matrix, tips were removed from their stems and placed into 1.5-mL microcentrifuge tubes. A 125-µL internal standard (IS) methotrexate-d3-triglutamate (MTXPG$_3$-d3) working solution (4 nmol/L) was added to each tube, and samples were agitated on a multitube vortex mixer for 30 min at 1200 rpm before adding 5 µL of 70% perchloric acid (Sigma Aldrich) to precipitate proteins (without removing the tips from their eluent). After centrifugation for 5 min at 18000g, 20 µL of extract supernatant was injected onto the LC-MS/MS system.

The sample treatment procedure for isolated RBC and anticoagulated venous blood derived from the sample treatment procedure was previously reported with small modifications (13). A 100-µL RBC lysate or lysed total venous blood sample was treated with 25 µL of perchloric acid (70%) in the presence of 600 µL of water containing IS (final concentration, 4 nmol/L MTXPG$_3$-d3). After centrifugation at 18000g for 5 min, 10 µL of extracted supernatant was injected onto the chromatographic system. The RBC sample treatment procedure was shown to be equivalent to the original sample treatment procedure validated in our clinical laboratory (13) (data not shown).

LC/MS/MS method

4-Amino-10-methylpteroyltriglutamic acid (MTXPG$_3$) was purchased as an ammonium salt from Schircks Laboratories. Stable isotope-labeled MTXPG$_3$, MTXPG$_3$-d3, was purchased from Toronto Research Chemicals and used as IS. Daily calibration standards were prepared by spiking MTX-free lysed whole blood with different concentrations of MTXPG$_3$ (final concentrations: 100, 50, 25, 10, and 5 nmol/L) before absorption onto VAMS samplers by holding the tips in contact with the liquid surface until saturation. Quality control (QC) samples were prepared from a lot of MTXPG$_3$ different from that used to prepare standards with final MTXPG$_3$ concentrations of 100, 25, and 5 nmol/L. Standard and QC VAMS tips were dried for at least 3 h in the dark at ambient temperature before processing.

Chromatographic separation was performed on an Accucore pentafluorophenyl (PFP) analytical column (2.1 × 50 mm, 2.6 µm) protected by an Accucore PFP Defender guard column (10 × 2.1 mm, 2.6 µm) (Thermo Fisher Scientific). The mobile phase consisted of 0.1% formic acid with 0.01% triethylamine and acetonitrile. The mass spectrometer consisted of a TSQ-Quantiva triple quadrupole mass spectrometer (Thermo Fisher Scientific) with a heated electrospray ionization source operating in the positive mode. Detection of MTXPG$_3$ and MTXPG$_3$-d$_3$ were performed at the m/z transitions of 713.23→308.09 and 716.36→311.13, respectively. Confirming ions were measured at the m/z transitions of

713.23→175.10 and 716.36→175.11, respectively. Run time was 6 min, and the retention times of both MTXPG$_3$ and IS were 2.3 min.

Intraday precision and accuracy was performed with 10 spiked replicates, whereas interday evaluation was assessed with 5 replicates on 5 different days. Accuracy was calculated as the percentage difference between the measured concentrations from each spiked sample relative to the target concentration. Precision was assessed by using the CV (%). Recoveries were determined as the peak area of MTXPG$_3$ recovered from MTX-free samples spiked with MTXPG$_3$ relative to the peak area of MTXPG$_3$ spiked directly into perchloric acid extracts. Limit of detection and limit of quantification were defined as the smallest concentration yielding a peak area signal-to-noise ratio of 5, and the smallest concentration yielding CV <15%, respectively. Calibration curves were calculated using quadratic regression (weighted $1/X^2$ and forced through the origin). Three level controls were used in each run.

Preanalytical experiments

Stability of MTXPG$_3$ collected on VAMS was tested 10 days after collection using clinical specimens collected from RA patients (with storage at ambient temperature). In addition, deidentified venipuncture-derived anticoagulated blood specimens received for RBC MTXPG$_3$ testing were absorbed onto VAMS tips to prepare dried blood specimens and evaluate the stability of MTXPG$_3$ for 30 days at ambient temperature. At each timepoint, VAMS specimens were extracted and MTXPG$_3$ was determined as described above.

Method comparison

For each patient enrolled, MTXPG$_3$ levels recovered from dried capillary or venipuncture-based blood fractions (total venous blood and isolated RBCs) were compared using ordinary least-squares linear regression (R^2, regression slope estimate, and intercept) and Deming regression. Regression analysis was also used to evaluate analyte stability. Acceptability criteria consisted of a slope ranging from 0.85 to 1.15 (15% bias) with $R^2 \geq 0.9$. Cohen's linearly weighted κ was used as appropriate.

Implementation in clinical practice

MTXPG$_3$ levels from capillary and venous blood specimens were ordered by physicians during clinical care of patients with rheumatic diseases. Patient information consisting of age, sex, and weekly MTX dose was provided on test requisitions at the discretion of the ordering clinician. All MTXPG$_3$ levels determined from dried capillary blood were reported as nanomole per liter RBC equivalent. A dose-normalized polyglutamation rate (PG rate) defined as the concentration of MTXPG$_3$ produced per milligram MTX administered (nmol/L/mg) was calculated. To maintain patient privacy, all specimens were deidentified before analysis. Differences between the distributions of dried capillary and venipuncture-based RBC MTXPG$_3$ levels and PG rates were assessed using the Kolmogorov–Smirnov (K-S) test. Differences in means were tested using the Mann–Whitney test. Potential nonadherence and gross underexposure to MTX was defined as long-chain MTXPG$_3$ levels <5 nmol/L RBCs.

RESULTS

LC-MS/MS analytical performance

Representative chromatograms of MTXPG$_3$ and MTXPG$_3$-d3 from calibrators and dried capillary blood specimens collected on VAMS tips from patients treated with MTX are presented in Fig. 1. Analytical performance is highlighted in Table 1. The method was linear between 5 and 100 nmol/L. Intraday and interday precision ranged from 2.0% to 10.9% and 3.1% to 10.8%, respectively, at concentrations ranging from 5 to 100 nmol/L. Mean
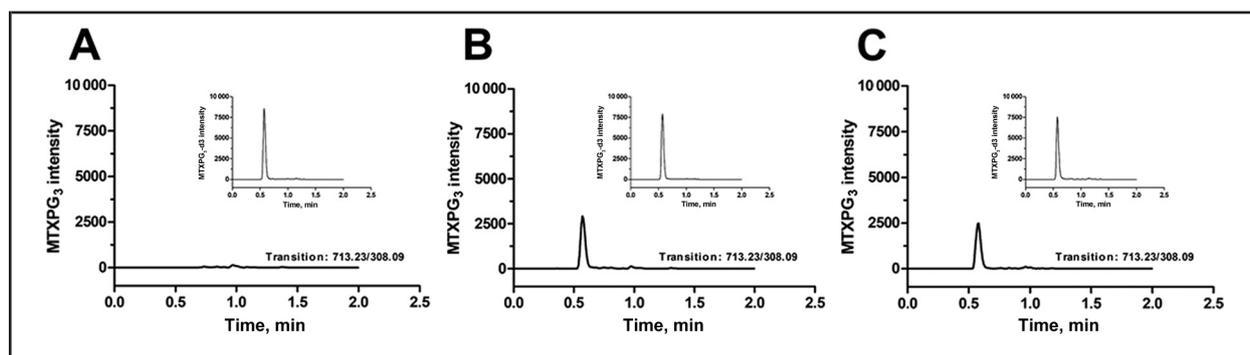
Fig. 1. Representative MTXPG$_3$ and IS chromatograms.
Typical chromatograms of MTXPG$_3$ and MTXPG$_3$-d3 (windowed) monitored at tandem mass spectrometry transitions of 713.23/308.09 and 716.36/311.33, respectively. (A), VAMS extract of blank dried anticoagulated whole blood. (B), 25 nmol/L QC. (C), VAMS containing capillary blood of an MTX-treated patient. The patient was 74 years old, was prescribed 17.5 mg of MTX per week, and had 24.0 nmol/L MTXPG$_3$ corresponding to 41 nmol/L RBC equivalent.

accuracy of the target value ranged from 89% to 106%, and recoveries were >80% at all target concentrations. The lower limit of quantification was 5 nmol/L, and the limit of detection was 1 nmol/L.

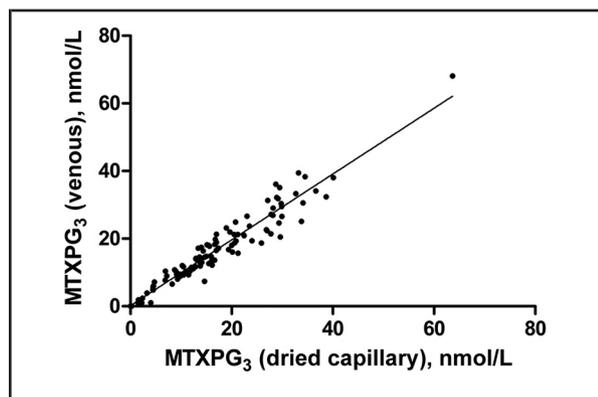### Method comparison: dried capillary blood and venous blood

In the 106 RA patients enrolled [age, 62 ± 13 (mean ± SD) years; 80% females; MTX dose, 17 ± 5 (mean ± SD) mg/week], MTXPG$_3$ levels recovered from anticoagulated venous and dried capillary blood specimens (3 days after collection) were both 17 ± 11 nmol/L (mean ± SD; range, 0–68 nmol/L and 0–64 nmol/L, respectively). Hematocrit was 0.42 ± 0.03 (mean ± SD; n = 106). Patient surveys collected for each patient are summarized in Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www. jalm.org/content/vol4/issue1, and revealed that the fingerstick collection method was convenient and noninferior to venipuncture, with a minority of participants (37%) being comfortable with self-collection of the capillary blood specimen at home. The correlation between MTXPG$_3$ levels recovered from venous anticoagulated whole blood and dried capillary blood specimens (Fig. 2) yielded a slope of 0.97 ± 0.03 (95% CI, 0.92–1.03) and an intercept of 0 (95% CI, −1 to 1) with a coefficient of determination (R^2) of 0.92, thereby indicating that the methods were comparable. Deming's slope was 1.02 ± 0.03 [95% CI, 0.96–1.07; intercept, −1 ± 1 (95% CI, −2 to 1)].

Table 1. Analytical performance of MTXPG$_3$ from dried capillary blood.

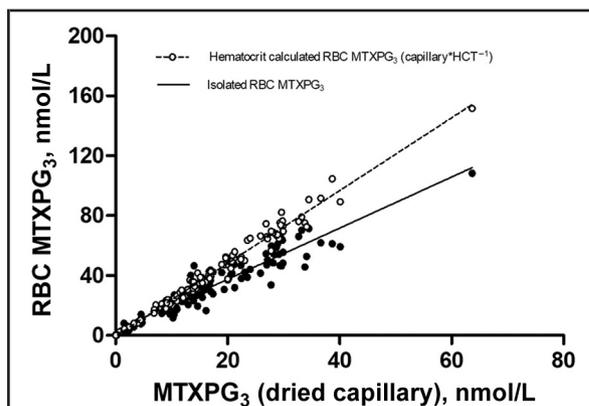| Target concentration, nmol/L	Intraday (n = 10)			Interday (n = 5)			Extraction	
	Mean observed concentration, nmol/L	Mean accuracy of target value, %	RSD[a], %	Mean observed concentration, nmol/L	Mean accuracy of target value, %	RSD, %	Recovery, %	RSD, %
5	5.6	88.9	10.9	5.4	92.8	10.8	86.3	0.3
25	23.1	108.0	5.9	23.8	105.1	5.3	88.0	2.0
100	98.6	101.4	2.0	100.4	99.6	3.1	88.4	13.8

[a] Relative standard deviation.

Fig. 2. Comparisons between MTXPG$_3$ levels recovered from venous blood and dried capillary blood.

Linear regression (solid line) between MTXPG$_3$ levels in venous blood and dried capillary blood collected on VAMS ($R^2 = 0.92$; n = 106); slope = 0.97 ± 0.03 (95% CI, 0.92–1.03), intercept = 0 ± 1 (95% CI, −1 to 1).

Fig. 3. Comparisons between MTXPG$_3$ levels recovered from dried capillary blood, and isolated RBCs.

Solid line shows correlation between MTXPG$_3$ levels recovered from dried capillary blood and isolated RBCs ($R^2 = 0.89$; n = 106); slope = 1.71 ± 0.05 (95% CI, 1.59–1.82), intercept = 3 ± 1 (95% CI, 1–6). Dashed line shows correlation between MTXPG$_3$ levels recovered from dried capillary blood and converted to RBC MTXPG$_3$ using 1/hematocrit (HCT) and isolated RBCs ($R^2 = 0.97$; n = 106); slope = 2.43 ± 0.03 (95% CI, 2.36–2.50), intercept = 0 ± 1 (95% CI, −2 to 2).

Method comparison: dried capillary blood, venous blood, and isolated RBCs

In the 106 RA patients enrolled, MTXPG$_3$ levels from isolated RBCs were 33 ± 19 nmol/L (mean ± SD; range, 0–108 nmol/L). The correlation between MTXPG$_3$ levels recovered from dried capillary blood and isolated RBCs is presented in Fig. 3. Slope estimates between venous and capillary blood and isolated RBC levels were 1.71 (95% CI, 1.61–1.81) and 1.71 (95% CI, 1.59–1.82), respectively ($R^2 = 0.9$). Deming's regression estimates are provided in Table 1 of the online Data Supplement. Calculation of RBC MTXPG$_3$ levels (RBC equivalent) from venous and dried capillary blood using a patient-specific 1/hematocrit conversion factor (mean ± SD; 2.4 ± 0.02) revealed an overestimation of MTXPG$_3$ levels by 1.31-fold (95% CI, 1.2–1.4; $R^2 = 0.92$) and 1.28-fold (95% CI, 1.2–1.4; $R^2 = 0.90$), respectively (Fig. 3) compared with isolated RBC MTXPG$_3$ levels. This finding was consistent with the 29% lower slope estimate observed between capillary blood and RBC levels (1.7 slope vs 2.4 hematocrit conversion factor). The cause for these higher MTXPG$_3$ levels in blood (total venous or capillary)

compared with isolated RBCs was unrelated to the presence of MTXPG$_3$ in plasma, which was undetected in the plasma of all patient specimens tested (data not shown).

We hypothesized that higher extraction recovery of MTXPG$_3$ in the presence of plasma (as seen in venous or capillary blood) in contrast to water (as seen during the sample treatment procedure of isolated RBC lysates) could be the cause for this overestimation. To test this hypothesis, we used 30 deidentified venous blood specimens (collected in EDTA) received for routine MTXPG$_3$ TDM in the clinical laboratory. Hematocrit was determined (mean = 0.43 ± 0.08) in each specimen before isolating RBCs. RBC lysates were resuspended to a final volume of 100 µL in MTX-free pooled plasma (to mimic whole blood matrix) or water (normally used to process RBC lysate) while respecting hematocrit proportionality for each

patient (i.e., 45 µL of RBC lysate was resuspended with 55 µL of water or plasma if hematocrit was 0.45), and subjected to the sample treatment procedure (addition of 600 µL of water followed by perchloric acid deproteinization). Mean (± SD) recovered $MTXPG_3$ levels were 1.28-fold higher in the presence of plasma compared with water (23 ± 10 nmol/L vs 18 ± 8 nmol/L isolated RBC), a finding consistent with the higher recovery of $MTXPG_3$ from venous and capillary blood compared with isolated RBCs, as described above.

Venous whole blood and dried capillary blood $MTXPG_3$ levels were converted to RBC equivalent (nmol/L) using a conversion factor (1.7, the slope estimate between dried capillary blood and isolated RBCs, as described above), and this resulted in comparable $MTXPG_3$ levels between isolated RBCs (33 ± 19 nmol/L), venous whole blood (29 ± 18 nmol/L RBC equivalent), and dried capillary blood (30 ± 18 nmol/L RBC equivalent) (slope = 0.9; 95% CI, 0.8–1.0). Cohen's weighted κ also revealed an acceptable agreement between collection methods, with cutoffs corresponding to subtherapeutic (<20 nmol/L; 9 venous vs 4 capillary specimens), intermediate (20–60 nmol/L; 66 venous vs 70 capillary specimens), and therapeutic levels of $MTXPG_3$ (>60 nmol/L; 31 venous vs 32 capillary specimens) (weighted κ = 0.72 ± 0.06; 95% CI, 0.60–0.84).
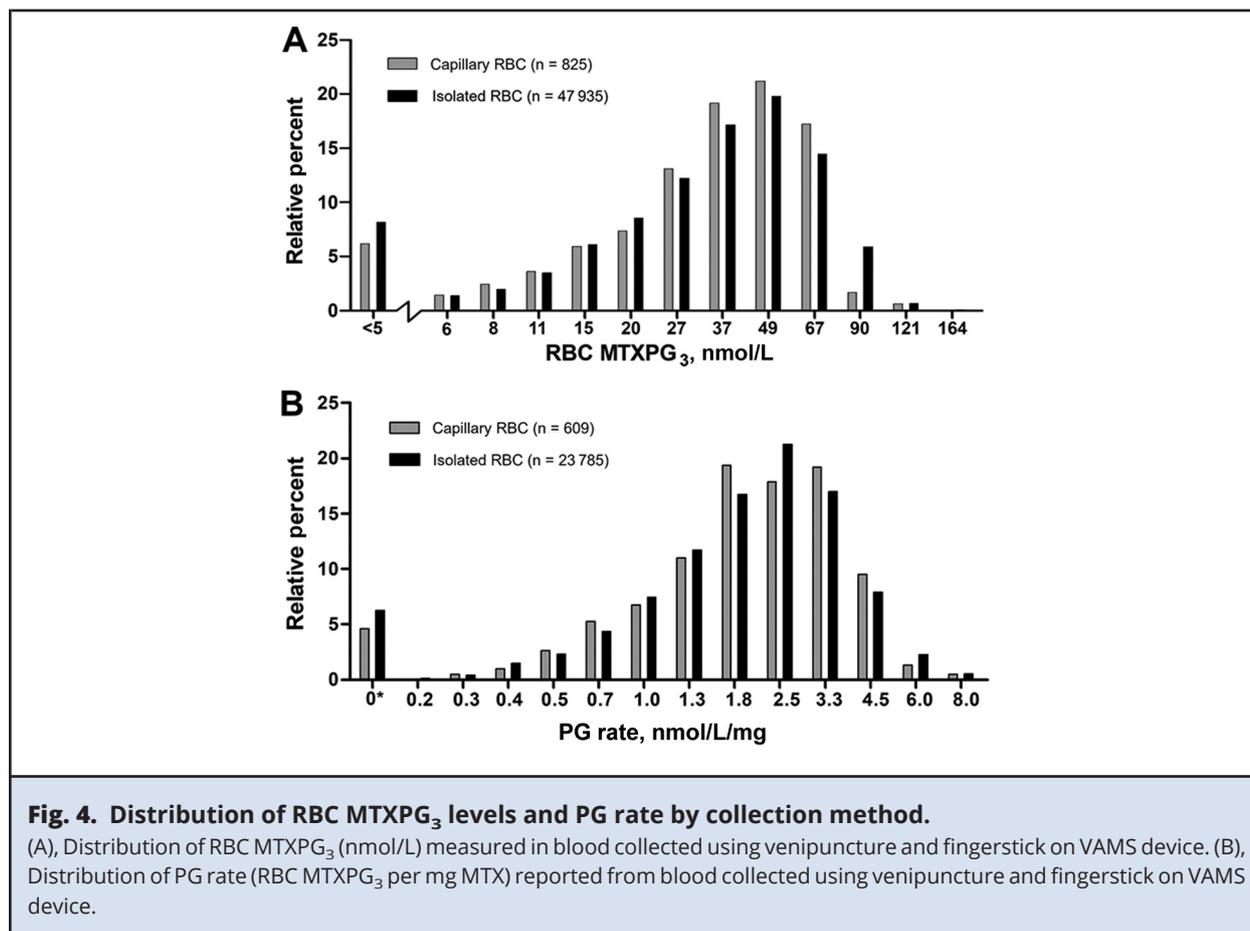
Preanalytical variations

In 38 RA patients, $MTXPG_3$ levels recovered from dried capillary blood specimens after 10 days (mean, 17 ± 7 nmol/L) were similar to levels recovered at baseline from whole blood (mean, 17 ± 7 nmol/L) (R^2 = 0.92; slope = 0.97; 95% CI, 0.9–1.1; n = 38). There was no difference in dried blood $MTXPG_3$ recovered after 30 days of storage at ambient temperature (mean, 19 ± 11 vs 21 ± 12 nmol/L; R^2 = 0.94; slope = 0.90; 95% CI, 0.8–1.0; n = 29). Results are presented in Fig. 1 of the online Data Supplement.

Implementation in clinical practice

Between January 2017 and February 2018, a total of 825 capillary blood specimens (n = 609 with MTX dosing) were collected for TDM from 679 patients (mean age, 58 ± 15 years) and compared with a database of 47935 venipuncture-based specimens collected from 35624 patients (mean age, 60 ± 15 years) (n = 24187 specimens with MTX dosing information available). Rejection of VAMS specimens was 4% (2% because of overfilling and 2% because of underfilling).

RBC $MTXPG_3$ estimates from capillary blood specimens (39 ± 22 nmol/L RBC equivalent, mean ± SD; n = 825) were comparable with those from isolated RBC specimens collected using venipuncture (39 ± 24 nmol/L; n = 47935) (Fig. 4A) (Mann–Whitney U P = 0.65) with no significant difference in the RBC $MTXPG_3$ distributions (K-S P = 0.09). Also, similar levels were observed between collection methods during the first year of testing [36 ± 21 nmol/L RBC equivalent (n = 768) vs 36 ± 21 nmol/L RBC (n = 5299)].

In the group of specimens submitted with dosing information, average weekly MTX dose provided on the test requisitions was slightly higher for specimens collected by venipuncture (18.5 ± 4.9) compared with the fingerstick method (17.8 ± 5.1 mg/week) (P < 0.010). Dose-normalized PG rate defined as the concentration of $MTXPG_3$ produced per milligram MTX administered was similar between the venipuncture-based method (2.25 ± 1.58 nmol/L/mg; n = 24187) and the fingerstick method (2.23 ± 2.06 nmol/L/mg; n = 609) (Mann–Whitney U P = 0.24) (Fig. 4B) with no significant difference in distribution between collection techniques (K-S P = 0.20). Potential poor compliance to MTX therapy (≤5 nmol/L RBC) was detected in 7.0% (n = 54) and 8.5% of specimens (n = 4224) collected using the fingerstick method and venipuncture, respectively.

Fig. 4. Distribution of RBC MTXPG$_3$ levels and PG rate by collection method.
(A), Distribution of RBC MTXPG$_3$ (nmol/L) measured in blood collected using venipuncture and fingerstick on VAMS device. (B), Distribution of PG rate (RBC MTXPG$_3$ per mg MTX) reported from blood collected using venipuncture and fingerstick on VAMS device.

DISCUSSION

We report a capillary blood-based method that can accurately quantify nanomolar concentrations of intracellular long-chain MTXPG$_3$ using at least 2 orders of magnitude lower blood volume than venipuncture. We chose to use a volumetric absorptive microsampling collection device that has the unique property to accurately collect 10 μL of blood from fingerstick, and coupled this collection method with a simple sample treatment procedure to quantify MTXPG$_3$ using high-sensitivity LC-MS/MS.

It was essential to establish the correlation and comparability of MTXPG$_3$ levels recovered from RBCs and those recovered from the capillary blood specimens, which are received in the laboratory

without any hematocrit estimates. During the development of the method, we observed that RBC MTXPG$_3$ levels calculated from anticoagulated whole blood using a hematocrit-based conversion factor (1/hematocrit) were 1.3-fold higher than those from isolated RBCs, and these data support findings from an earlier report featuring specimens collected on dried blood spot cards (21). The higher levels recovered from the total blood matrix were unrelated to MTXPGs from plasma, which were undetectable, or white blood cells, which accumulate negligible MTXPGs relative to RBCs, as shown (22) (T. Dervieux, personal communication). Rather, our results strongly suggest that greater extraction efficiency of MTXPG$_3$ from RBCs in the presence of plasma and its constituents during sample treatment procedure introduce an

overestimation of RBC levels. Caution should be exercised while interpreting RBC $MTXPG_3$ levels produced by a hematocrit-based back calculation when a similar sample treatment procedure to that used here [first presented by Dervieux et al. (13)] is used, and potential for overestimation should be taken into account.

Therefore, it was expected that a much lower conversion factor (1.3-fold lower) than the hematocrit-based conversion (1.7 vs 2.4) would be applicable to convert $MTXPG_3$ recovered from capillary blood to RBC equivalent. Applying a 1.7 conversion factor yielded an acceptable comparison between fingerstick and venous blood levels, indicating the 2 collection methods could be used interchangeably in TDM practice. We also collected a patient survey, which revealed that specimen collection via fingerstick was noninferior to venipuncture with respect to patient convenience. However, two-thirds of patients were unsure or uncomfortable with collecting capillary blood specimens at home, and we do not recommend the self-collection of specimens at this point, at least in patients with RA.

Measurement of RBC $MTXPG_3$ levels can help detect underlying causes of a lack of response to MTX—primarily nonadherence to therapy, poor absorption, or accelerated excretion, all of which potentially contribute to inadequate MTX exposure and, thus, poor disease control. After 1 year of testing in our clinical laboratory, we compared RBC equivalent $MTXPG_3$ levels measured in dried capillary specimens with a large database of nearly 50000 specimens collected for $MTXPG_3$ assessments from RBCs isolated from blood collected using standard venipuncture. Our data revealed similar distributions of RBC $MTXPG_3$ and similar dose-normalized levels between the fingerstick-based and venipuncture-based collection methods, a finding consistent with the degree of correlation achieved during the clinical validation study. The analysis also revealed that a proportion of patients prescribed MTX presented with very low RBC MTXPG levels (≤5 nmol/L), and these data illustrate potential for noncompliance to treatment (23) or, alternatively, compliance in the context of one or more of poor absorption, increased excretion, and poor activation of MTX to its active polyglutamated metabolites.

In conclusion, we have developed a capillary blood-based method to quantify long-chain $MTXPG_3$ in adult patients with rheumatoid disease and transitioned that method to the TDM laboratory. Moving forward, this method can be extended to the TDM of low-dose MTX therapy in other specialties, including pediatrics (24).

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